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The effect of water content in soil media on the hatchability of *Bractocera dorsalis* pupae

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Abstract. Fruit flies are polyphagous pests on various important fruit crops. The *Bactrocera* genus, including *Bactrocera dorsalis* (Hendel), undergoes a pupation process in the soil. Efforts to control this species in the pupal phase can be done by applying entomopathogenic fungi. Entomopathogenic fungi need a moist environment to grow optimally. This study aims to evaluate the effect of adding water to soil media on the success of *B. dorsalis* pupae becoming imago. The soil media used comes from orange fields. Soil media is sterilized first before being used to test the success of fruit fly pupation. The addition of water was carried out with the following water and media content ratios: 0 : 1, 0.075 : 1, 0.15 : 1, 0.184 : 1, 0.225 : 1, 0.3 : 1 and 0.5 :1. The pupa is placed in a mica tube with a diameter of 5 cm and poured with granulated soil, so that it reaches a height of 4 cm. Replication was carried out four times. The parameters observed include success in becoming an imago, survival, and normality. The highest percentage of pupae that hatched was found at a water to soil addition ratio of 0.15 to 1 (86.25% ± 5.54). The highest percentage of living imago was also at the same water addition concentration (27.5% ± 1.44), as was the percentage of normal imago (81.25% ± 5.90). However, the death rate at this concentration was also high (58.75& ± 4.73). The highest percentage of abnormal pupae was found in media without the addition of water (0: 1). There was a tendency for success rates to become imago, survival, and normality to decrease at higher water concentrations. The findings of the regression analysis show the survival equation y = -4.33x + 28.75. No pupae successfully hatched at a water concentration of 0.5 to 1 v/v. At concentration after two days, the soil becomes dry and hard to clot. This study recommends using a water concentration of 0.15 to 1 v/v for applications with entomopathogenic fungi to create sufficient soil moisture.

1 Introduction

Fruit flies are one of the important pest groups in fruit and vegetable commodities. One species with important economic value is *Bactrocera dorsalis*, which can attack various types of fruit and vegetable commodities [1-3]. Control of *B. dorsalis* is relatively tricky, the female

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imago lays eggs in the fruit so that the eggs hatch and grow in the fruit. After the final instar stage, the larvae bounce out of the fruit. The larvae bury themselves and transform into pupae when falling to the ground. After 6 to 7 days, the pupae hatch into adults, and the adult fruit flies burrow higher into the ground [4]. To overcome the problem of fruit fly attacks, farmers generally carry out control during the adult phase. Examples include the use of methyl eugenol, modified traps, pollen baits, sterile insect approaches, and botanical insecticides [5–8]. Fruit fly control can, in principle, be done at all stages, including larvae or pupae. Pupae that live and develop in the soil depend highly on soil conditions, including moisture levels. Water content determines the success of the pupation process. At extremely high and low levels, pupae can fail to become imago. Pupae have preferences for soil moisture levels. Previous research shows that the best soil moisture is found in 10–60% conditions, producing imago emergence rates exceeding 90% at the moisture levels [9]. Soil moisture is also important for controlling pupae using entomopathogenic fungi.

Beberapa jenis entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill., *Isaria fumosorosea* Wize, *Metarhizium robertsii* JF Bisch, SA Rehner & Humber, and *Glomus* spp.sudah banyak dikaji pengaruhnya terhadap pupa lalat buah. A previous study reported that the moisture level in our experiment to 50% (wt/wt), is optimal for survival. In laboratory tests, the mortality of larvae and pupae of *B. carambolae* was 70%. In addition, 100% of the adults who emerged died within five days after the emergency [10]. The water humidity level affects mold's viability, because mold can grow well in a humid environment.

In contrast, preliminary tests were carried out in the study using a water and soil ratio of 1: 1 (50%) v/v and 0.5: 1 (33.3%) v/v, demonstrating zero imagos hatched because the soil became hard and clumped. This study suspects that soil preparation by grinding the soil into small particles causes clumping soil after it was mixed with water and dried. Therefore, the optimal water content was tested using a soil depth of 4 cm. In previous studies, soil depth affected the success of pupae becoming imago, and imago success was optimum at 0 – 4 soil depth. Therefore, this study adopted that soil depth. This study aims to evaluate the effect of adding water to soil media on the success of *B. dorsalis* pupae becoming imago.

2 Materials and methods

In this study, the larval phase of *B. dorsalis* fruit flies was collected from rotten red chili (*Capsicum annum*) fruits from Sumberejo and Pesanggrahan Villages, Batu District. The characteristics of the chilies that were taken are those that are wrinkled and have traces of fruit fly ovipositor punctures. The red chilies were transferred and maintained in the Animal Diversity Laboratory, Department of Biology, Brawijaya University, Indonesia. Larvae were maintained in plastic boxes equipped with tissue and left until they became pupae. After becoming pupae, this phase of the fruit fly was taken for treatment. The treatment was carried out in the laboratory under normal conditions without microclimate factor conditioning. Laboratory conditions were maintained at room temperature (22-33°C) with a photoperiod of 14:10 (light : dark). The experimental process refers to previous research [11]. This study used soil collected from Dau District, Malang Regency, East Java, as an experimental medium. The samples were taken from two locations of orange gardens (mixed with chili) using a hoe. After being in the laboratory, the two soils were mixed and left for 48 hours until dry. The dry soil was pounded and sieved using a 2 mm sieve. The soil was put into 1 kg plastic and then sterilized in an autoclave at 121ºC for 15 minutes. Some of the soil was then tested physically for its texture. The test results showed that the soil consisted of clay (72%), sand (10%), and silt (18%).

The treatments tested in this study were the ratio of water and soil volume. Five treatments of water and soil ratios were tested using volume/volume ratio, including 0.075:1 (7%), 0.15:1 (13%), 0.184:1 (15.5%), 0.225:1 (18.4%), 0.3:1 (23%) and 0.5:1 (33.3) v/v. Air-dried soil without water (ratio 0:1) was used as a comparison or control. The soil was mixed with water in a sterile basin, and the treatment was carried out following the following procedure. Experiments were carried out by placing pupae in soil media. Soil media was put into transparent plastic and formed into a tube with a diameter of 5 cm. Approximately 10 cm of space was added to the height of the tube to ensure oxygen availability. The plastic tube is filled with soil media 1 cm thick. After that, the pupa is placed on top, and then soil is poured into the tube. Each tube consisted of 20 individuals of pupa and was repeated four times. The top of the tube was covered with a layer of cotton cloth to prevent adult fruit flies from escaping. After 6 days, pupa status in each treatment was checked every day until 16 days after treatment.

The number of adults released and their physical condition were observed. Fruit flies are classified based on the perfection of their abdomen and wings. Imago with deformed or flawed wings are considered abnormal. The number of adults hatching, survival, and morphological normality from each treatment was calculated, and the result was presented as a percentage, while the length of time development was presented as day. Data normality analysis was carried out. The results indicated that all parameters were not normally distributed. Furthermore, all transformation methods failed to normalize the data. Therefore, statistical analysis was suitable for non-parametric analysis. When the effect of parameters was significant, the post hoc tests were carried out using the Kruskall-Wallis test. All statistical tests were processed using SPSS® version 18, and the results of the tests were considered different when $P<0.05$.

3 Results and discussions

The highest percentage of pupae that successfully hatched into imago was found at a water content of 13% (86.25% ± 5.54). The highest percentage of surviving imago was also at the same water content (27.5 ± 1.44) and the percentage of normal imago (81.25% ± 5.90). However, the death rate at this concentration was also high (58.75 ± 4.73). The highest percentage of abnormal pupae was found in control or media without water addition (20 ± 2.04). There was a tendency for imago success, survival, and normality to decrease at higher water concentrations. At a water content of 33%, there were no pupae successfully hatched (Table 1).

Table 1. Effect of water treatment (%) in the soil media on imago success, survival, death, abnormal and normal.

Parameters	Water content (%)						
	0	7	13	15.5	18.4	23	33.3
Imago success (%)	57.5 ±5.95b	21.25 ±21.25a	86.25 ±5.54b	5±5.00a	2.5±2.50a	6.25±6.25a	0a
Survival (%)	26.25 ±3.15b	13.75 ±13.75ab	27.5 ±1.44b	5±5.00a	2.5±2.50a	5±5a	0a
Death (%)	31.25 ±3.15bc	7.5 ±7.5ab	58.75 ±4.73c	0a	0a	1.25±1.25a	0a
Normal (%)	37.5 ±5.95b	21.25 ±21.25a	81.25 ±5.91b	5±5a	1.25±1.25a	6.25±6.25a	0a
Abnormal (%)	20 ±2.04 b	0 a	5 ±2.04 ab	0 a	1.25±1.25a	0 a	0a

Note: value followed by different alphabet indicated the significantly different means

Based on statistical analysis, the treatment of water addition had a significant effect on the imago success (χ^2 = 18.793; P < 0.01), survival (χ^2= 16.761; P < 001), pupa death (χ^2 =

22.415; P < 0.01), normal pupa (χ^2 = 18.520; P < 0.01) and abnormal pupa (χ^2 = 21.120; P < 0.01) (Table 2). Furthermore, the relationship between the treatment of water addition (variable x) and the survival rate of imago (variable y) was tested using regression analysis, where the findings showed the survival equation y = -4.33x + 28.75 (Fig. 1). This result indicated that high water levels influence high mortality rates.

Table 2. Statistical analysis of the effect of water addition to soil media on success, survival, death, normal and abnormal imago.

Test	Imago success	Survival	Death	Normal	Abnormal
Kruskal-Wallis H	18.793	16.761	22.415	18.520	21.120
Asymp. Sig.	0.005	0.010	0.001	0.005	0.002

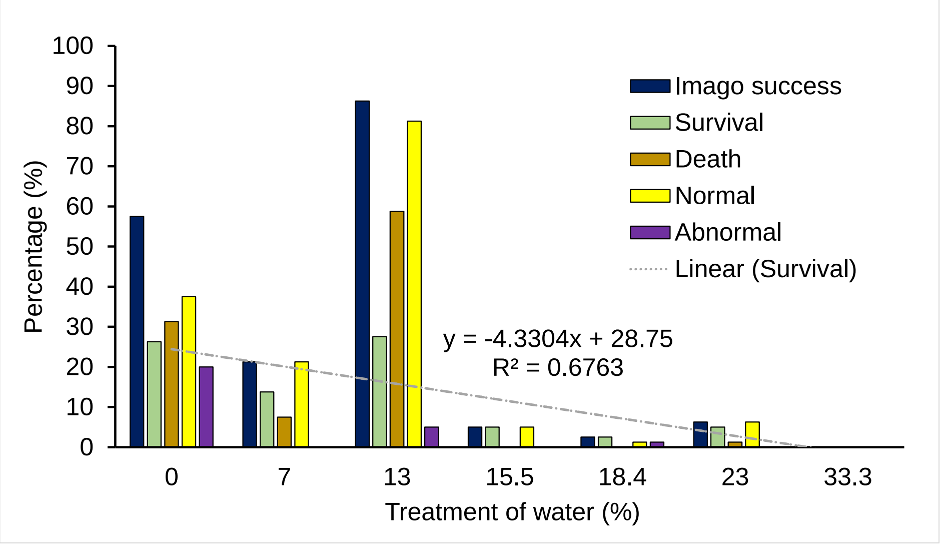

Fig. 1. The percentage of *B. dorsalis* emergence, survival and fatality in various water and soil ratios.

From the observations of fruit flies appearing on the surface of the soil on mica tubes, it was seen that there were deformed fruit flies. Compared to normal flies, which have fully developed wings, bright body color, and long stomachs (Figure 2A). The appearance of young, deformed fruit flies has the characteristics of a pale-yellow body, wrinkled wings, and a short, small abdomen. The horizontal black lines and longitudinal median lines are barely visible (Figure 2B). Defects in the pupa generally cause imago disorders and death.

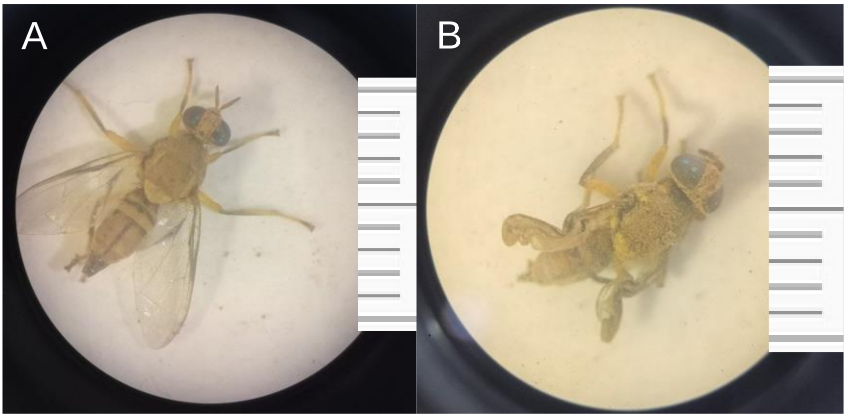

Fig. 2. Normal (A) and abnormal (B) imago.

The average pupal development time ranged from 7 to 16 days. The fastest pupal development time occurred at a water content of 18.4%. The longest pupal development time occurred at a water content of 23%. The average pupal development time in the control was the fastest at 8.09 ± 0.30 days, but none of the imago that managed to emerge from the soil survived. The average pupal development time that successfully became imago was 8.44 ± 0.24 days. The average pupal development time that successfully became a surviving imago was 8.81 ± 0.37. The average pupal development time that was the longest occurred at the abnormal imago 8.83 ± 0.60. In the water content of 7%, the average pupal development time was the fastest at 7.17 ± 0.00. This occurred in the imago, which successfully emerged from the soil without any survival. The slowest average pupal development time occurred in surviving pupae (10.55 ± 0.00). The average development time of pupae that successfully became imago and normal pupae was the same, namely 9.35 ± 0.0. In the water content of 13%, the fastest average pupal development time was 7.60 ± 0.38. This occurred in imagoes that successfully survived. The slowest average pupal development time occurred in abnormal imago (9.67±1.15) (Table 3).

Table 3. The development time (day after treatment) of *B. dorsalis* pupae

Parameters	Water treatment (%)						
	0	7	13	15.5	18.4	23	33.3
Imago success	8.44±0.24	9.35±0.00	8.79±0.48	15.50 ±0.00	7.00±0.00	14.60 ±0.00	N/A
Survival	8.81±0.37	10.55±0.00	7.60±0.38	15.50 ±0.00	7.00±0.00	14.25 ±0.00	N/A
Death	8.09±0.30bc	7.17±0.00ad	9.38±0.55b	N/A	N/A	16.00 ±0.00cd	N/A
Normal	8.25±0.14	9.35±0.00	8.75±0.47	15.50 ±0.00	7.00±0.00	14.60 ±0.00	N/A
Abnormal	8.83±0.60b	N/A	9.67±1.15ab	N/A	7.00±0.00a	N/A	N/A

Note: N/A = imago is absent

Based on statistical analysis, the treatment of water addition had a significant effect on the imago death (χ^2 = 19.994; P < 0.01) and abnormal pupa (χ^2 = 20.291; P < 0.01). It did not affect imago success (χ^2 = 11.934; P > 0.05), survival (χ^2= 11.320; P > 0.05), normal imago (χ^2 = 11.922; P > 0.05) (Table 4).

Table 4. Statistical analysis of the effect of water addition to soil media on average pupa development.

Test	Imago success	Survival	Death	Normal	Abnormal
Kruskal-Wallis H	11.934	11.320	19.994	11.922	20.291
Asymp. Sig.	0.063	0.079	0.003	0.064	0.002

Generally, *B. dorsalis* larvae pupate below the soil surface. This study indicates that the most optimal water and soil ratio for pupal development is 13% (0.15:1 v/v). At this ratio, the percentage of successful imago reaches more than 86%, and 27% survive. In a previous study, it was reported that pupae develop optimally in conditions of 10–60% moisture levels. In this study, the soil depth used was 4 cm. Another study reported that *B. dorsalis* fruit flies can emerge at a depth of 0–2 cm, and the soil moisture level ranges from 30–70% had a survival rate reaching 81%. In this study, the average emergence of imago began to decline at a water and soil ratio of 15.5% (0.184: 1 v/v) to 23% (0.3: 1 v/v), while at a water content of 33%, no imago successfully emerged to the soil surface. At a water and soil ratio of 0.5: 1, the soil became clumped and hardened after a few days, so the imago failed to penetrate it. The highest percentage of abnormal imago occurred at a water content of 0% (control). In the control, 20% of imago experienced abnormal symptoms. In general, abnormalities in adult flies were found in their wings. The imago fruit fly's wing growth was imperfect, shrivelled or wrinkled (Figure 2B). The other characteristic was swollen abdomens. Physical and chemical characteristics of the soil, such as interstitial space, grain size, compaction, water content, and organic matter content, can affect the emergence of adult fruit flies on the soil surface. The success of fruit fly larval pupae increased in soil with large soil particles because adult fruit flies easily emerged from the pupa. Several soil properties, such as porosity and total organic matter, can reduce soil density and make it easier for fruit flies to emerge. Conversely, fine soil has low porosity, and when exposed to water, it becomes lumpy, making it difficult for imago to emerge from the pupa and emerge to the soil surface.

The characteristics of the fine soil inhibit adult fruit flies from moving upwards toward the soil surface when the soil is mixed with water. In hardened soil with high density, adults have to work harder to reach the surface due to the low soil porosity [12]. This can slow down the time required for adults to emerge to the surface, thus prolonging the development time compared to the control. Good soil porosity allows for optimal soil drainage so adults can easily exit to the surface. However, abnormal growth in some adult fruit flies indicates that specific soil depths may exceed the limits of normal imago development capacity. Factors such as soil porosity, texture, density, humidity, and temperature affect pupal mortality rates and the emergence of deformed flies [12]. Other studies have shown that soil texture significantly affects the survival of *Bactrocera dorsalis* pupae and soil moisture levels [13, 14]. The results of this study also underline the importance of not throwing rotten fruit into the soil carelessly without proper management. If rotten fruit is buried at a depth of less than 50 cm, the soil must be covered to prevent flies from emerging to the surface. We recommend that farmers collect rotten fruits attacked by fruit flies and put them in closed plastic. Later, the fruit can be processed as fertilizer after 1 week.

The impact of soil moisture on the development of this species is not fully understood. However, other studies have reported that *B. dorsalis* tends to metamorphose in moist soil compared to dry soil [15]. In that study, the soil was categorized as "moist" at a relative saturation of 58% and "dry" at 27% [15]. In addition to soil moisture, pupal media and other environmental factors also affect the mortality rate of fruit flies. This study showed that the development of larvae into pupae can be inhibited when soil moisture reaches 70%. Larvae and/or pupae are likely to die in soil with high moisture, such as the soil in this experiment which has a field capacity of 65.1%. Previous studies have also found that pupae fail to survive in soil that is too dry or too wet [9]. In addition, pupae at 30% soil moisture are known to emerge faster than other humidity levels. To achieve humidity of 10, 20, and 30%, the soil in this experiment was mixed evenly with water, which changed the soil's physical properties. Soil pore size increases, and soil density decreases, making it easier for pupae to move to the surface [9].

4 Conclusion

This study shows that the highest percentage of hatched pupae was found at a water content of 13% (86.25 + 5.54). The highest percentage of living imago was also at the same water addition concentration (27.5 + 1.44) and the percentage of normal imago (81.25 + 5.90). However, the death rate at this concentration was also high (58.75 + 4.73). The highest percentage of abnormal pupae was found in the control (0% water addition). There was a tendency for success rates to become imago, survival, and normality to decrease at higher water concentrations. The findings of the regression analysis show the survival equation $y = -4.33x + 28.75$. At a water concentration of 33%, no pupae successfully hatched. At concentration, after two days, the soil becomes dry and hard to clot. This study recommends using a water concentration of 13% for applications with entomopathogenic fungi to create sufficient soil moisture.

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